

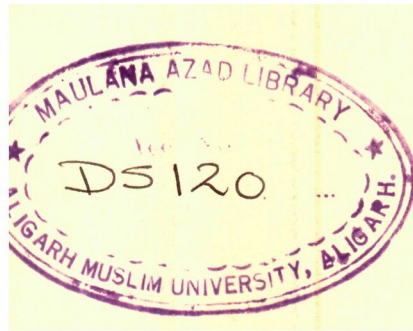


**STUDIES ON POWDERY MILDEWS—
Powdery Mildew of Legumes**

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INTRODUCTION AND REVIEW OF LITERATURE

Legumes are cultivated all over the world and stand next to cereals in their economic importance. According to Allen and Allen (1958) legumes as a whole form a very important group of plants because they are not only source of high quality of proteins but they also provide adequate quantities of minerals and vitamins. They are also essential in well-balanced crop rotation programmes. The legumes are grown in all the states of India either as a crop or mixed cropping. Out of these, pulses alone are grown in 60 million acres of land which is one-sixth of the total cropped area and one-fifth of the area under food grains.

The yield of legumes per acre in India is far below than the similar standard in agriculturally advanced countries. This is partly due to the fact that a large proportion is taken up by plant pests and pathogens like bacteria, viruses and fungi etc. Of the different pathogens attacking legumes, powdery mildew is of no less importance. Beaumont et al. (1928) reported that pea crop in Southwest of England suffered heavy losses due to Erysiphe polygoni De Caudolle ex Saint-Amans. Vasurat et al. (1960, 1965) observed 25-40 per cent loss of clovers due to E. polygoni in Germany whereas Pozhar (1961) and Trofimets

(1962) reported 60 percent losses on different crops in irrigated areas of South Ukraine and USSR, respectively. In India, Uppal et al. (1935) reported that powdery mildew resulted in the reduction of number of pods picking from seven to one in heavily infected fields. According to Vasudeva (1960) there was a loss of 25 percent in pea pod yield in powdery mildew infected fields. Munjal et al. (1963) assessed 21-31 percent reduction in pod number and 26-47 percent in pod weight.

Powdery mildews form a clearly defined group of Ascomycetous fungi and are characterized by their superficial white mycelium, the production of large, colourless (or white) non-septate, turgid, air-borne conidia, their diurnal periodicity with respect to several characters, the reversible phototropism of some species, the compatible association with their hosts and their vulnerability to control by fungicides.

Although powdery mildews as a group were recognized by Linnaeus as early as 1753, but according to Saemann et al. (1952) the order Perisporiales includes two families viz., Erysiphaceae and Perisporiaceae. The latter is clearly distinguished from the former by their dark mycelium, being lesser parasitic and their different types of conidiophores. The Erysiphaceae, on the basis of degree of parasitism, is divided by Salmon (1900) into two subfamilies, Erysiphinae and Phyllactiniaceae and by Homa (1937) into three subfamilies, Erysiphinae, Phyllactiniaceae and Leveilluleae.

Powdery mildews as a pathogen of legumes attack the plantation every year throughout the country. The entire plant is covered with white powdery mass which affects not only the yield but also the quality. Yarwood (1954) also reported that infection by B. polygoni appeared on all the parts of leaves of bean except pulvini. Infected leaves show yellowing or chlorosis (Cook, 1931 and Parris, 1941) followed by browning of infected cells (Shiraishi et al., 1976) and ultimately resulting into their premature falling (Parris, 1941 and Yarwood, 1951).

Histological symptoms include changes in staining; properties of infected cells (Leon; et al., 1970), collapse of the necrotic epidermal cells and tissues below the penetrated epidermal cells and movement of host nuclei towards the haustoria (Smith, 1938). In resistant varieties the development of infection hyphae is arrested (Rubin, 1956). Smith (1938), Hilitzer (1938), Rubin (1956) and Stavely et al. (1966) reported that necrosis of the penetrated epidermal cells and even of adjacent cells followed by collapse of the cells are characteristic reactions of plants' resistance to powdery mildew. Jhooty et al. (1967) reported that more powdery mildew lesions were formed when leaves were subjected to mechanical pressure before heating.

Powdery mildew infections also change the physiology of the plants by increasing transpiration (Hilitzer, 1938;

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and Ayres, 1976), especially at night (Yarwood, 1936); respiration (Yarwood, 1934, 1953 and Latch et al., 1968) and decrease in the photosynthesis (Parris, 1941 and Ayres, 1976). Decreased cellulose content (Morse et al., 1969) and acidifying system (Rubin, 1956); Increase in protein content (Sturm, 1958), peroxidase and Glucose-5-phosphate dehydrogenase bonds (Stavely et al., 1967) and pisatin production (Cruickshank et al., 1960; Oku et al., 1975 and Shiraishi et al., 1976) as a result of infection have also been reported.

IDENTITY OF THE PATHOGEN

The identity of the powdery mildew fungus attacking legumes as a whole and pea in particular had been a subject of controversy. Although De Candolle (1805) and Greville (1825) reported that the pathogen of powdery mildew of pea was Erysiphe pisi but Salmon (1900) remarked that E. polygoni as such was most variable species of the Erysiphaceae and suggested that it was not desirable to separate these variations into separate species. He thus synonymised E. pisi with E. polygoni. Blodgett (1915) and Weiss (1950), on the other hand, have assigned the fungus as E. polygoni and Microspheera alni as well. Homma (1937) and Yarwood (1957) reported that the confusion in the two powdery mildew fungi was mainly due to the apparent similarity of the conidiophores of E. polygoni and M. alni.

Formation of perithecia on pea led Da Silva et al., (1966), Jensen (1967), Griffe (1969) and Parmelee (1977) to confirm that powdery mildew on pea was E. polygoni. Bouwens (1924, 1927), however, identified powdery mildew of pea as E. polygoni on the basis of conidial characters. Hammarlund (1925), later suggested that it was E. polygoni f.sp. pisi and Yu (1946) designated it as E. polygoni DC. f. viciae pisi n.f. In 1949, Oidium erysiphoides has been reported on peas (Anonymous). Palti and Stettiner (1957) reported Oidiopsis and E. polygoni affecting pea plants during the maturity of the crop. Parmelee (1977) and McLaughlin et al. (1977) have identified Microspheera diffusa on Glycine max. and Kharbanda et al. (1977) have identified Microspheera penicillata var. ludens on Vicia faba and several Lathyrus spp. Golovin (1956) observed that Erysiphe communis f. sp. betae attacked Phaseolus mungo and other legumes. Erysiphe martii has been reported attacking Melilotus officinalis (Viennot-Bourgin, 1958); Melilotus sp., Lathyrus sp., Trigonella sp., Psoralea sp., Albizia sp. and Astragalus sp. (Blumer 1967); Trifolium alexandrinum (Mayor, 1967) and Trifolium sp. (Tomson, 1934). Erysiphe trifolii has also been reported on legumes (Pall et al., 1968; Kapoor, 1967 and Parmelee, 1977); Leveillula leumincosarum on Medicago spp., Psoralea sp. and Vicia tenuifolia (Blumer, 1967) and f.sp. medicaginis on Medicago falcata (Bysova, 1961). Bysova (1961) observed Erysiphe cichoracearum f.sp. trifolii on Trifolium pratense and E. cichoracearum f.sp.

medicaginis on M. falcata whereas E. communis f.sp. medicaginis has been reported on lucerne by Daroshkin et al. (1976) and E. communis f.sp. trifolii on T. pratense by Jovanovic (1969).

Sphaerotheca fuliginea has been found attacking Astragalus alpinus (Blumer, 1967 and Parmelee, 1977); Phaseolus radiatus (Uozumi et al., 1952); Vigna sinensis, Phaseolus vulgaris, Rhynchosia acuminatifolia (Hirata, 1955) and Cyamopsis tetragonoloba, Dolichos uniflorus, P. vulgaris, P. lathyroides, Vigna vexillata, V. lanceolata and Glycine javanica (Alcorn, 1968,69). Yarwood (1963), 1965) suggested that both S. fuliginea and E. cichoracearum attacked P. vulgaris but Tai (1936) observed S. humuli var. fuliginea on P. vulgaris and P. mungo. Diaz and Pilomena (1963) reported that E. partii (E. cichoracearum) attacked most of the legumes.

Hammarlund (1925) as reported by Blumer (1967) held E. pisi a valid species and divided it into four physiologic forms viz., 2. sp. pisi on P. sativum and P. arvensis, f.sp. medicaginis-sativae on M. falcata and M. sativa, f.sp. medicaginis-lupulinae on Medicago lupulina and f.sp. viciae-sativae on Vicia sativa, V. sepium and V. silvatica. Hirata (1955) has also separated E. polygoni into various species and identified E. pisi on wild pea on the basis of type of conidial germination. Golubev (1965) and Kotova et al. (1965) observed that powdery mildew of pea was E. communis f. sp. pisi (E. polygoni). Later Nikolaeva (1968)

reported that E. communis also attacked large number of legumes. Bulbakova et al. (1971) confirming the above observations suggested that E. communis f.sp. pisi attacked peas. Blumer (1933, 1967), Hirata (1955), Junell (1967) and Kapoor (1967) identified the powdery mildew of pea as E. pisi. Blumer (1967) has attempted to separate E. pisi from E. polygoni on the basis of presence of fewer and rarely branched appendages on the perithecia in the former as against abundant and irregularly branched appendages in the latter. Gram et al. (1922), Mayor (1947), Boelema (1963), Fuchs et al. (1971), Gorska-Poczopko (1971), Dongo et al. (1972), Khan et al. (1977), Gorter et al. (1974) and Nomura (1974) have reported that peas are attacked by E. pisi and not E. polygoni. Hirata (1956, 1976), while cataloguing the geographical distribution of powdery mildews, listed E. pisi attacking most of the legumes. Parmelee (1977) suggested that E. polygoni will be more appropriate for powdery mildew of legumes including pea in view of several overlapping characters between E. pisi and E. polygoni.

From India there are conflicting reports of the powdery mildews of legumes. Leveillula taurica has been reported attacking Dolichos lablab (Uppal et al., 1936) and M. sativa, C. tetragonoloba, Cajanus cajan and Crotolaria spp. (Patel et al., 1949) whereas Cidiosia taurica has been reported on Trigonella foenum-graecum (Uppal, 1935) and on Tephrosia tenuis (Mitter et al., 1930). Phyllactinia subspiralis has also been reported on

Dalbergia sissoo (Butler and Bisby, 1931) and Cassia obtusifolia (Uppal et al., 1935) but Pandotra et al. (1964) suggested that powdery mildew on another species of Cassia i.e., C. occidentalis was Sphaerotheca cassiae. S. humuli var. fuliginea has also been found attacking Rhassolus aconitifolius (Anonymous, 1950) and S. fuliginea on P. vulgaris (Vasudeva, 1960). Sohi et al. (1966) observed Sphaerotheca macularis on P. mungo.

It, therefore, appears that it is not one single powdery mildew fungus that attacks the legumes but there are several species and even genera which form the causal organisms of powdery mildew of different legumes. This confusion has probably resulted because in nature seldom perithecia have been observed on powdery mildew infected leaves. The perithecial production has rarely been observed in western part of Uttar Pradesh, whereas they have been frequently observed in East Uttar Pradesh (Saxena- Personal communication, Venkatakrisnaia, 1951 and Butler et al., 1931).

Salmon (1900) while listing 357 species in 157 genera suggested that E. polygoni has a widest host range than any other species. Under laboratory conditions the host range of E. polygoni has been studied by Salmon (1903) Searle (1920), Hains (1923), Hammarlund (1925), Blumer (1933), Yarwood (1936) and Kreitlow (1948).

Parmelee (1977) following Salmon's broad species concept reported E. polygoni on 31 species in 21 genera of 11 families in the province of Ontario, Canada. Blumer (1967) while assigning powdery mildew of pea to E. pisi listed about 48 species in 10 genera as hosts from Central Europe. Junell (1967) considered E. pisi as pathogenic on pea and gave a list of hosts in Sweden.

From amongst the legumes, E. polygoni has been reported on Phaseolus aureus (Litzenberger et al., 1957 & Soria et al., 1973), P. vulgaris (Whetzel, 1922; Gilvie, 1926; Zaunmeyer, 1930; Cunningham, 1931; Dundas, 1941; Yarwood 1950; De LaRocha, 1955; Litzenberger et al., 1957; Vieira, 1961; Crispin et al., 1964; Issa et al., 1964 and Diaz Polanco et al., 1966), P. lunatus (Snyder et al., 1945), P. coccineus (Yarwood, 1947), P. radiatus (Wallace, 1930), Lens esculenta (Wallace, 1936), V. sinensis (McDonald, 1937; Wallace, 1941 and FontisVidela, 1953), G. max (Lohman, 1931 and Johnson et al., 1943) and Medicago cordata and Saxifraga fortunei var. incisulobata (Hirata, 1955).

After Blumer's (1967) host list of E. pisi, Indigofera decora, Desmodium oldhami, D. racemosum, Lathyrus odoratus, Leopaeza virgata, Cassia noronae, Kummerowia striata, Amphicarpaea edgeworthii var. japonica, Weigela hortensis and W. decora have been added as hosts by Hirata (1955); Vicia faba by Rayss (1953) and Lupinus polyphyllus by Strukchinkas (1977).

In India, A. polygoni has been reported on P. mungo var. radiatus, Sesbania aegyptiaca and Vigna catjang (Patel et al., 1949); Trigonella polycerata (Grover, 1952); L. odoratus (Pavri et al., 1966); L. esculenta (Sankhla et al., 1967); P. mungo (Athur et al., 1971) and P. aureus (Gupta et al., 1976).

ENVIRONMENTAL FACTORS

There are several environmental factors which influence the development of powdery mildews and of these temperature and moisture have been reported to attribute a considerable effect on their development.

Knight (1918), Yu (1946), Yarwood (1949, 1950), Shands et al. (1964) and Kuppel et al. (1975) reported greater incidence of A. polygoni under dry than in wet conditions. Taking this into consideration Schmiedeknecht (1960) has designated powdery mildews as Xerophilic while Ayres (1977) reported that in case of peas the mycelial growth and spore production decreased when leaf-water potential decreased from -4.5 ± 0.5 bar (Soil water at field capacity) to -9.5 ± 1.0 bar (to the permanent wilting point). Similar results have been observed by Sturm (1958) with A. polygoni on clover. Poor development of powdery mildew during rains has been attributed not only to the mechanical damage to powdery mildew conidia (Yarwood, 1936), but also due to poor dispersal of conidia in the air during rainy weather (Yarwood, 1934), the greater occurrence of mildew parasite, Cicinnobolus

cesatii (Yarwood, 1957). The greater production of spores under moderate than under high relative humidities and their greater capability to germinate under low than under high atmospheric humidity (Hannarlund, 1925) have also contributed towards low development of powdery mildew during rains. In contrary to this, Yarwood (1936) showed that relative humidity of the atmosphere has had either no or slight effect on powdery mildew development. Yarwood (1936), Brodie et al. (1942), Brodie (1945) and Kothari et al. (1972) reported that conidia might germinate in relative humidities approaching zero (0% RH) which was because of high water content of the conidia i.e. 69 percent (Jhooty et al., 1965) and slow rate of water evaporation (Yarwood, 1952). Yarwood (1936) and Brodie (1945) reported that the germination of conidia of A. polygoni from Trifolium, Phaseolus ^{per cent} Delphinium and Oenothera was more at zero/relative humidity as compared to those from Brassica sp. It probably indicates that the host on which the conidia are formed also affect the capability of conidia to germinate at zero percent relative humidity. Kothari et al. (1972) observed that conidia of A. polygoni from poppy germinated at 100 percent relative humidity, but Stavely et al. (1966) found that the optimum relative humidity for germination was 52 percent. Yarwood (1936) and Kyryk et al. (1972) concluded that the germination was highest at 100 percent RH. However, the optimum relative humidity for development of pathogen and severity of disease was reported to be 70-80 percent by

Kyryk et al. (1972) and 85 percent with 1.61 inches rainfall (Soria et al., 1973). Yarwood (1936) reported that mycelium grew well at 0-100 RH, but low relative humidity followed by high temperature killed the fungus. Hammarlund (1925) reported that in damp and still air, the conidiophores of E. polygoni on Pisum sativum produced numerous chains instead of one conidium at the tip.

The minimum temperature for the germination of conidia of E. polygoni on cowpeas was 7°C (Paulech, 1969) and on clover 16°C (Stavely et al., 1966). The maximum temperature for conidial germination of cowpea strain was 33°C (Paulech, 1969), clover strain 28°C (Stavely et al., 1966) and poppy strain 32.5°C (Kothari et al., 1972). Kyryk et al., (1972) reported optimum temperature for the germination of conidia of E. communis f.sp. pisi to be 20°C whereas according to Stavely et al. (1966), Paulech (1969) and Kothari et al. (1972) it ranged from 20-25°C. Yarwood (1954) in his ^{excellent} review of literature, suggested that tolerance of powdery mildew to heat was usually lower than that of their hosts, and it was possible by treating the host to free plants of mildew without host injury. With E. polygoni on beans, this was possible in about 20 minutes at 40°C, 2 minutes at 45°C, 20 seconds at 50°C and 3 seconds at 55°C temperature. He later (1956) reported that when bean leaves were immersed in hot water for a few seconds before inoculations, their susceptibility to E. polygoni

was increased. Yarwood (1936) reported that heating of conidia of E. polygoni before germination increased germination.

Hammarlund (1925) observed that optimum temperature for the development of powdery mildew of pea was 20°C, whereas Kyrk et al. (1972) reported it to be as 20-25°C. Soria et al. (1973) reported optimum temperature to be 25.6°C for Congo strain.

Buchheim et al. (1928) reported that an average daily temperature of 310 to 320°C from the moment of first appearance of conidia of E. polygoni on Caryana orboreasceus was most conducive to the production of perithecia. Smith (1970), on the other hand, reported that 10-20°C temperature was favourable for the initiation of cleistocarp of E. polygoni of pea and Lathyrus ochroleucus.

Hammarlund (1925) and Yarwood (1934) suggested that light is essential for the development of powdery mildew in general. Soria et al. (1973) observed that for severe development of E. polygoni on P. aureus, 9976 g-cal-cm²-day total solar energy is required. Kothari et al. (1972) however, reported that germination of conidia was unaffected by light and dark. Yarwood (1957) reported that severity of mildew of plants increased with the duration of light upto a certain point and then decreased. He in 1942 observed more luxuriant development of powdery mildew in shade than in full natural light. Light may affect powdery mildew both directly affecting the fungus or indirectly through

its effect on the host. Yarwood (1934) reported that in low light intensities or darkness, photosynthesis is reduced which ultimately reduced the carbohydrate supply to fungus. Yarwood (1936, 1957) further observed that when some portion of petiole of infected bean plants were darkened, the diurnal periodicity of conidiophore maturation of A. polyzoni was not apparent after five days on the darkened portion while rest of the part was showing diurnal periodicity.

Uspenskaya (1958) reported that the disease incidence was not only high but the disease appeared earlier in poorly cultivated soils whereas it was delayed in well cultivated soils. Nijngaarden et al. (1969) reported that development of powdery mildew was reduced when ammonium nitrate was supplied to the plants. The infection was reduced by high potassium (Sturm, 1958). Tret'yakova et al. (1965) observed that powdery mildew infection was reduced by high level of P_2O_5 and K_2O and bacterial fertilizers (phosphobacterin and nitragin). Thompson et al. (1976) found that there was an increase in the development of powdery mildew at higher dose of KH_2PO_4 and low dose of $Ca(NO_3)_2$. Higher dose of silicon (Lowig, 1935) and boron (Yarwood, 1938) resulted in poor development of the mildew.

Brown (1930) observed that high soil pH favoured the development of mildew on cowpea. However, Yarwood (1931) obtained

no such effect from a graded series of application of sodium hydroxide and sulphuric acid to soil.

Considerable work has been done on the control of powdery mildews by the use of chemicals including inorganic and organic sulphur (carbamate) fungicides, copper sulphate preparations, cuprous oxide preparations, organic mercurial compounds, various inorganic compounds, systemic compounds like captan, benlate, aureofungin, griseofulvin, olivomycin, and cercobin etc., antibiotics, organic dyes and mineral oils (Yarwood, 1946, 1951, 1957 and del et al., 1974).

Feichtmeir (1949) reported that toxicity of sulphur may be increased by higher temperature. Monroe (1963) while assaying the inhibition of spore germination of S. polygoni in vitro and in vivo by ten fungicides, observed different results with different fungicides.

Compounds like copper carbonate and karathane have been found more effective than sulphur dust (Durrell, 1931 and Starker, 1954). Bonnet et al. (1962) and Chencogne (1962) have maintained that binasacryl (depaacryl) and crotonate compounds were ten times and five times more fungicidal than wettable sulphur, respectively. On the other hand, Kontaxis (1976) reported that sulphur dust was superior to wettable sulphur, benmoyl, curic hydroxide or three proprietary fungicides.

Seeds treated with certain chemicals have resulted into lesser development of powdery mildew of different legumes (Crawford, 1927; Kivi, 1963; Proshaev, 1964; Mikhaileenko, 1965; Chancogne et al., 1969; Arif, et al., 1970 and Jhooly et al., 1972). Tomlinson et al. (1958, 1959 and 1960) have controlled the disease by adjusting the mineral composition of the soil. Dekker (1961), El-zayat et al. (1967), El-zayat et al. (1968) and Hammett (1968) have used systemic fungicides for an effective control of powdery mildew whereas Nel et al. (1974) have used antibiotics and Gorter et al. (1974) purine and pyrimidine compounds for the control of E. pisi.

In India also various inorganic and organic compounds like sulphur dust, wettable sulphur, cosan, elosal, karathane, thiovit, sulkol, morestan, morocide, aureofungin, perenox, sandhakghol, sultaf, daconil-2737, bordeaux mixture, thiophanate compounds, tridemorph, sulfex and calixin have been used to control powdery mildew of legumes (Joshi, 1955; Srivastava et al. 1973; Pathur et al., 1971; Naish et al., 1970; Gupta et al., 1976; Ghose et al., 1966; Pathur et al., 1972; Singh et al., 1974; Singh et al., 1975; Khare et al., 1974; Ghose et al., 1970; Chatur et al., 1977; Rathi et al., 1977 and Shukla et al., 1977).

Ayres (1977) observed that mycelial growth and spore production of E. pisi decreased when soil was not watered before or after inoculation of the plants. When kolodust was applied immediately after rain or a water spray and then allowed to dry,

it adhered much longer than did ordinary flowers of sulphur (Anonymous,, 1930). Alicbusan et al. (1959) suggested that application of fungicides in the morning, when spray drift was less in the stiller air, helped to prevent the big release of spores round about midday.

The injurious action of rain on powdery mildew (Yarwood, 1934, 1936) has naturally suggested the possibility of combating diseases by means of a water spray and Yarwood in 1939 observed that colonies of A. polygoni on P. vulgaris were reduced from 1600 to 20 per four plants, when sprayed daily, at about 70 lb. pressure in the later afternoon, for 11 days, starting at 24 hours after inoculations. Boughay (1949) has shown that the incidence of powdery mildews decreased as the rainfall increased. Spraying of plants with hot water, combining the inhibiting effect of water and heat effectively controlled the powdery mildew but the treatment has greater risk of plant injury (Torbrun, 1949 and Anonymous, 1899).

Although sincere attempts have been made by several workers to develop varieties of legumes resistant to powdery mildew fungus, but none of them could maintain the resistance for quite a long time (Kolp, 1952; Webb et al., 1961 and Aray et al., 1975).

It is clear from the brief review of literature that no systematic work has been carried out in India on the powdery

mildew of legumes. Moreover, there appears to be more than one species or even genera involved in the causation of the disease. Nothing is known as to why the powdery mildew generally appears in the later part of growing season of the crop. Hence in order to understand the above aspects of parasitism of powdery mildew of legumes the following studies will be made.

- I. The field in and around Aligarh will be surveyed to assess the incidence of powdery mildew on different legumes and to identify them as far as possible.
- II. Effect of different temperatures and relative humidities on germination of conidia of powdery mildew from different legumes.
- III. Effect of different temperatures and relative humidities on the development of powdery mildew on different hosts.
- IV. The host range of powdery mildew from different legumes.
- V. Screening of different cultivars of peas and cowpeas against respective powdery mildews.
- VI. Effect of Boron on the development of powdery mildew of pea.
- VII. Phyllosphere mycoflora , both of healthy and diseased plants.
- VIII. Effect of Nematicides and Oil-cakes on the development of powdery mildew on pea.

MATERIALS AND METHODS

SURVEY

Fields with Leguminous crops in and around Aligarh will be visited round the year to record the incidence and severity of the powdery mildew. The four meter square area will be selected randomly in each field and number of plants showing symptoms will be counted and the degree of severity and percentage of infection will be determined. Three such readings will be taken per field. In the kitchen gardens the disease intensity will be determined in the entire bed.

The severity of powdery mildew will be graded as under-

No infection	(-)	=	No macroscopically visible diseased symptoms.
ild infection	(+)	=	Pustules few, small in size and scattered.
oderate infection	(++)	=	Pustules many, larger in size, tending to coalesce.
Severe infection	(+++)	=	Big pustules covering almost the entire leaf area, also on stem and fruits.

IDENTITY OF LEAF POWDERY MILDEW

Attempts will be made to base the identification of powdery mildew on cleistothecial characters as far as possible.

In case of their non-availability the conical characters will be taken into consideration.

For identifying the powdery mildew fungus the infected leaves from different plants will be collected from various localities and will be brought to the laboratory in polythene bags. In order to maintain the culture in the glasshouse the healthy seedlings, of the plant from which the material has been collected, will be inoculated periodically so that inoculum is available for further studies.

Seeds of different plants will be surface sterilized and later sown in autoclaved soil. Seedlings in the cotyledonous stage or 3 leaf stage and at times leaves of mature plants will be inoculated by dry dusting technique as proposed by Schmitt (1959).

From the infection thus developed the mycelial and conidial characters viz., colour of the mycelium in older pustules (Modigh, 1936 and Yarwood, 1957), shape (Alcorn, 1963, 1969 and Blumer, 1967) and size (Louwens, 1924, 1927; Blumer, 1967; Kapoor, 1967; Junell, 1967 and Parmelee, 1977) of conidia, presence and absence of fibrosin bodies (Rosen, 1937; Clare, 1958, 1964; Kable et al., 1963 and Jhoopty, 1967) and type of germ tube during the germination of conidia (Virata, 1942, 1955; Kable et al., 1963 and Karacovitis, 1965) will be studied.

For locating the fibrosis bodies under microscope, conidia will be mounted in 3 percent solution of A.R (Able, et al., 1963) and will be examined under the microscope.

For studying the type of germ tube, formed during the germination, the conidia will be dusted over dry clean glass slides placed on glass - triangle in a petri-dish with double distilled water at the bottom. These will later be transferred in an incubator running at 17-22°C. After 24 hours the conidia will be stained in cotton blue and will be mounted in lactophenol and the type of germ tube formed will be observed.

For determining the size, the conidia will be stained in cotton blue and mounted in lactophenol. In each case, about 250-300 conidia will be measured. The size so obtained will be subjected to statistical analysis.

The size and shape of cleistothecia, number of asci per cleistothecium and number of ascospores in each ascus will also be determined.

In addition to this the various other components of cleistothecia will be measured.

EFFECT OF DIFFERENT TEMPERATURES AND RELATIVE HUMIDITIES ON GERMINATION OF CONIDIA AND DEVELOPMENT OF DISEASE

(a) Germination of Conidia

Freshly collected conidia of almost same age will be

dusted over the dry clean slides with the help of a glass-rod (Fair et al., 1962). These slides will be kept on glass-triangles placed in petridishes containing double distilled water at the bottom. These will be transferred to incubators each running at -5, 5, 10, 15, 20, 25, 30 and 35°C respectively.

Super saturated solutions of different salts will be used for maintaining different relative humidities (Table 1).

Super saturated solutions of	Relative humidities % at 20°C
Calcium chloride anhydrous	0
Chromium trioxide	35
Sodium nitrate	66
Sodium acetate	76
Ammonium sulphate	81
Zinc sulphate	90
di-Sodium hydrogen phosphate	95
Copper sulphate	98
Double distilled water	100

(Hand book of Physics and Chemistry, 1957).

The dessicators will be used as chambers. The glass slides containing conidia will be placed on glass-triangles kept above the level of solutions in the dessicators. The entire assembly will be kept at 20°C.

The germination of the conidia will be studied after 4, 8, 12, 24, 36 48, 60 and 72 hours of incubation. Percentage of germination will be calculated by counting the total number of conidia in three different fields and those that will germinate.

(b) Development of powdery mildew

In order to determine the effect of different temperatures and relative humidities on the appearance and severity of the powdery mildew and cleistothecial production, surface sterilized seeds of C. tetragonoloba (L.) Taub., L. esculenta Moench., P. sativum L., T. foeniculaceum L., V. faba Peterm., Vigna aurea (L.) Wilczek and V. mungo (L). Hepper will be sown in autoclaved soil contained in 15 cm clay pots. At the seedling stage the plants will be inoculated with the powdery mildew. Inoculated plants will immediately be transferred to growth chamber running at 5, 10, 15, 20, 22, 25, 30 and 35°C respectively. At each of the above temperatures the effect of 50, 60, 70, 80, 90 and 95 percent relative humidities will be studied. The inoculated plants will be regularly examined for the appearance of the powdery mildew. Inoculated plants will be left in the chamber for about a month or so to look for the development of cleistothecia.

The effect of different temperatures and relative humidities on the development of powdery mildew of legumes will also be studied on detached leaves or leaf discs (Morrison, 1960,64).

Leaves will be removed from the uppermost nodes of uninfected plants. Discs will be cut with 1 cm diameter sterilized cork borer and will be floated on sterilized water contained in sterilized petri-dishes. Detached leaves, on the otherhand, will be placed on glass slides kept on a glass-triangle in a petridish with the petiole dipped in water. They will then be inoculated with the conidia from different hosts and will be examined regularly for the production of symptoms.

Seedlings of different legumes will also be inoculated in different parts of their growing season and the development of disease will be examined. The plants so inoculated will be left till the fruiting in order to observe the production of cleistothecia.

HOST RANGE AND HOST SPECIALIZATION

For host range studies seedlings of C. cajan (L.) Willsp., C. occidentalis L., Cicer arietinum L., C. tetragonoloba, D. lablab L., L. esculenta, L. sativa L., L. coccineus L., L. lunatus L., P. vulgaris L., P. sativum, Trifolium alexandrinum L., T. repens L., T. foenum-graecum, V. faba, Vigna acconitifolia (Jacq.) Marechal, V. aurea, V. mungo and V. unguiculata (L.) Walp. will be raised in autoclaved soil, contained in 15 cm claypots. When cotyledons have emerged the seedlings will be inoculated with conidia of powdery mildew from different legumes. Inoculated plants will then be transferred to separate glasshouse chamber and will

regularly be examined for the appearance of the disease.

For studying the varietal resistance different cultivars of pea and cowpea will be grown in autoclaved soil, contained in 15 cm clay pots and will be inoculated with powdery mildew conidia from their respective hosts.

The inoculated plants will be transferred to glasshouse bench or the field as the case may be.

EFFECT OF BORON

Seeds of most susceptible variety of pea will be sown in acid leached and thoroughly washed river sand, contained in 15 cm glazed crockes. To each pot boron free Long Ashton nutrient solution (Hewitt, 1966) will be added daily. The seedlings will be inoculated with pea powdery mildew at three-leaf stage. To six sets of pots, each replicated five times, will be added 0.5, 1.0, 2.5, 5.0, 7.5 and 10.0 ml solution having 5, 10, 25, 50, 75 and 100 ppm boron respectively except the seventh set of pots which could serve as control.

After 20 days of inoculations the plants will be uprooted, washed and dried in hot air even at 60°C. water soluble boron will be estimated in sands of different pots and total boron content will also be estimated in roots and shoots (Berger and Group, 1944). For water soluble boron 10 g oven-dried sand will

be taken and for roots and shoots finely grinded, oven-dried plant material (0.5 g) from different treatments will be placed separately in porcelain dishes. These will be ignited to a white or grey ash over an open flame. The remaining contents will be cooled and 10 ml of 0.1N HCl will be added in the dish. The residue will then be triturated and filtered. From each filtrate 1.0 ml aliquot will be taken in polythene beakers and 4.0 ml curcumin:oxalic acid reagent will be added. Again the aliquots will be dried in an oven at $55 \pm 3^{\circ}\text{C}$. The resocyanine colour will develop. It will again be cooled and dissolved in 25 ml of 95 per cent ethyl alcohol. Absorbance will be recorded in Bausch & Lomb Spectronic - 20 Colorimeter at 580 m.m.

Standard curve of boron will be prepared by taking 2, 1, 3, 4, 5, 6 and 7 ml. of 10 ppm of B/ml solutions in 50 ml standard flask and will be made upto mark with the conductivity water. Then 1 ml from each solution will be taken in 100 ml polythene beaker and 4 ml of curcumin:oxalic acid reagent will be added to each beaker. These two solutions will be mixed well and dried at $55 \pm 3^{\circ}\text{C}$ and the residue will be kept at this temperature for 15 minutes to ensure dryness. The coloured substance, resocyanine will develop during the evaporation and drying. The beakers containing the dried residue will be cooled to room temperature. Then 25 ml of 95 percent ethyl alcohol will be added to each beaker, the residue will be triturated to extract the colour and the solution will be filtered through a whatmann No. 42 filter

paper directly to the calorimeter tube. The colour will be read with a 580 m.m. light maximum within two hours.

Reference will be made to the calibration curve corresponding to the maximum light employed for the determination and carbon (in microgram) contained in the 1 ml of aliquot will thus be determined.

PHYLLOSHERE MYCOFLORA

For studying phyllosphere mycoflora seeds of resistant and susceptible varieties of pea will be sown in autoclaved soil, contained in 25 cm clay pots. The pots will be placed near each other to ensure similar climatic conditions. The collections will be made after 15, 30, 45, 60 and 90 days of seedling emergence to determine the phyllosphere mycoflora. Leaf washing technique will be used (Nickinson, 1967). One of the lower most leaflets will be picked, on each occasion, from each of the ten plants. The leaflets will be washed and stirred thoroughly in 10 ml of sterilized water. The washings will be plated out in 10 petri-dishes containing Potato Dextrose Agar plus streptomycin. The fungi that appear, will be isolated and identified.

EFFECT OF NEMATOCIDES - CIL CROUS

It has been observed in nature that plants grown in soil treated with nematocides became relatively free to powdery mildew. Hence the seedlings of P. sativum will be raised in soil treated

with Furadan 33 and Rogor & nematocides and ground nut, neem, mahua and mustard oil-cakes and will be inoculated with respective powdery mildew. Similar number of seedlings raised in untreated soil will be inoculated to serve as control.

RECORD OF DATA

Unless otherwise stated, the observations on the development of powdery mildew will be taken after 20 days of inoculation. The disease intensity will be rated as follows (Wheeler, 1969).

<u>Grade</u>	<u>Description</u>	<u>Infection type</u>
Highly resistant	Plants completely free from the infection	0
Resistant	Mycelium developing in small patches, disappearing later or at best covering 1-25% leaf area.	1
Moderately resistant	Mycelium developing both on leaves and stem covering 26-50% leaf-area.	2
Susceptible	Many small colonies appearing, later coalescing and covering 51-75% leaf-area. Mycelium developing on stem as well.	3
Highly susceptible	Entire plant covered uniformly by mildew.	4

The data will be subjected to statistical analysis. There will be five replicates of each treatment throughout the studies.

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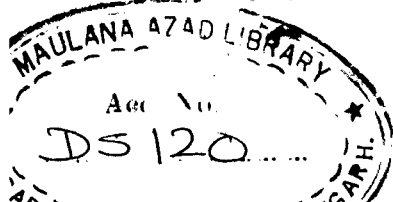
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